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THE CONFORMATION OF (+)-MUSCARINE IN SOLUTION

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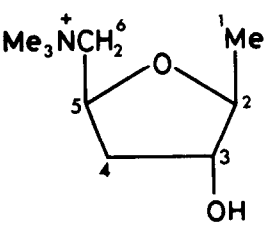
Abstract - An ^{1}H n.m.r. study of (+)-muscarine 1 in deuterium oxide solution shows that the t conformation 4 is highly favoured for the exocyclic C(5)-C(6) bond. Ring proton coupling constants can be accounted for on the basis of rapidly interconverting puckered forms largely favouring the C(4)-endo conformation.

Muscarine 1 has played a central role in neuropharmacology because of its strong and specific cholinomimetic activity.[1] This has generated a considerable interest in its conformation.[2] The crystal structure of (+)-muscarine iodide[3] bears a close resemblance to that of lactoylcholine chloride[4] and to the preferred conformation of acetylcholine in solution.[5] There is no information on the solution conformation of muscarine except for an ^{1}H n.m.r. n.O.e. investigation of the conformation about the C(5)-C(6) bond.[6] We have now carried out such a study using ^{1}H n.m.r. data on D_2O solutions of pure (+)-muscarine chloride,[7] essentially by utilising the close structure similarity with 2'-deoxynucleosides 2. There is much work, both in the N- and C-nucleoside series on which to draw.[8-13]



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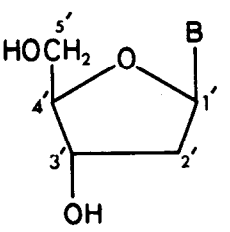


2.

The chemical shift data were obtained using a Varian XL100 spectrometer at 100 MHz and are collected in Table 1. Line assignments were made by decoupling studies and the coupling constants are given in Table 2.

Table 1 Proton Chemical Shifts (δ) (ppm)[a] for (+)-muscarine chloride

H-2	H-3	H-4'	H-4	H-5	H-6'	H-6	Me	$\overset{+}{N}Me_3$
3.90[b]	3.93	1.84	1.96	4.49	3.30	3.46	1.07	3.04

[a] Measured at 20° in D_2O solution with acetonitrile as internal standard; in the case of geminal protons, that resonating at higher field is designated with a prime.

[b] Approximate value.

Table 2 Coupling Constants (Hz) for (+)-muscarine chloride

$J_{1,2}$	$J_{2,3}$	$J_{3,4'}$	$J_{3,4}$	$J_{4',4}$	$J_{4',5}$	$J_{4,5}$
6.8	3.0	5.2	2.4	14.0	9.2	7.0

$J_{5,6'}$	$J_{5,6}$	$J_{6',6}$
8.3	3.0	13.5

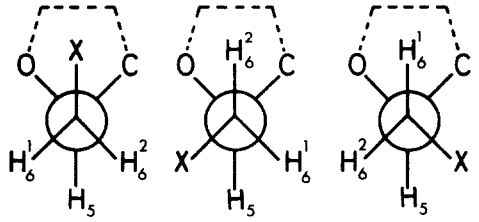
We consider first the rotamer preference about the C(5)-C(6) bond in 1. Established procedures are available for analysing the conformational preferences about the C(4')-C(5') bond in nucleosides and nucleotides, using $J_{4',5'}$ and $J_{4',5}$ " values.[12,13] Following this work we utilise corresponding equations

1 - 3 to analyse the muscarine system in terms of the populations (p) of the three staggered conformers 3, 4 and 5 ($X = \overset{+}{N}Me_3$).

$$J^1_{5,6} = p_+J^1_+ + p_aJ^1_a + p_-J^1_- \quad (1)$$

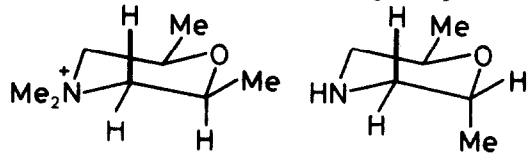
$$J^2_{5,6} = p_+J^2_+ + p_aJ^2_a + p_-J^2_- \quad (2)$$

$$1 = p_+ + p_a + p_- \quad (3)$$



3. 4. 5.

The evaluation of p_+,p_a and p_- depends on the specific assignment of the observed coupling constants to particular methylene protons. Following the corresponding assignments for nucleosides and nucleotides[8,12], $J_{5,6}$ and $J_{5,6'}$ are assigned respectively to $J^1_{5,6}$ and $J^2_{5,6}$. For muscarine J_+ and J_a values were obtained from the model compounds 6 and 7[14,15] assuming a negligible electronegativity difference between N^+ and N (J^1_+, 4.8; J^2_+, 1.3; J^1_a, 2.1; J^2_a, 11.2). The J-values were evaluated as for nucleosides[12] by using



6. 7.

the expressions of Abraham and Gatti,[16] for disubstituted ethanes, assuming a negligible electronegativity difference between C and H (J^1_-, 12.4; J^2_-, 5.4).

The conformation populations obtained by this analysis are given in Table 3. There is evidently a strong preference for muscarine to exist in the t conformation (4), confirming

Table 3 Conformer populations about the C(5)-C(6) bond in muscarine

p_+	p_a	p_-
0.04	0.88	0.08

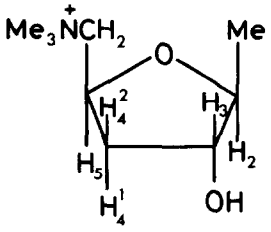
the earlier conclusion from n.O.e. studies by de Fontaine et al.[6] This is in line, too, with the crystal structure, also in the synclinal conformation, where the OCCN torsion angle is 73°.[3] Nottali, Lambert and Letsinger have shown that a 5'-amino-5'-deoxynucleoside has the same conformation (4; $X = NH_2$) about the C(4')-C(5') bond.[17] This is in marked contrast to the nucleosides and 5'-nucleotides which virtually exclusively have the g^+ conformation as in (3: X = OH or OPO_3H_2).

It is noteworthy that in the n.m.r. spectrum of muscarine the H-5 resonance is shifted markedly downfield compared with the H-4' resonance in nucleosides. The deshielding is presumably due to the magnetic anisotropic effect induced by the positive charge,[15] or to steric compression by the large trimethylammonio group in this conformation.[18] The H-5 resonance alone is broadened due to a relatively large NCCH coupling.[15]

In the crystal, the 5-membered ring of muscarine exists in a C(4)-endo,^{4}E, conformation, but no information is available on the solution conformation. Generally speaking tetrahydrofuran rings exist in dynamic equilibrium between two favoured puckered conformations and Altona and Sundralingam introduced a pseudorotational analysis whereby the 5-membered ring conformation could be calculated from observed vicinal coupling constants. The observed ribo-nucleoside and -nucleotide spectra were interpreted in terms of two ring conformers (C(2')-endo and C(3')-endo). With an electronegativity correction (1.1 Hz) to compensate for the replacement of OH by H at C(2') the method was extended to the 2'-deoxyribosyl derivatives. Davies and Danyluk[11] derived the relation 4 by a related scheme in which X_S and X_N are the mole fractions of the C(2')-endo and C(3')-endo conformers respectively and showed that the equilibrium values for a series of deoxynucleosides 2 agreed well with those derived by a complete pseudorotational analysis. Before analysing the coupling constants of the ring protons

$$K_{eq} = X_S/X_N = J_{1',2'}/J_{3',4'} \quad (4)$$

of muscarine it is necessary to assign the 4- and 4'-signals. Following the argument of Davies and Danyluk[11] based on coupling constant magnitudes, the high field signal H-4' was assigned to H^2_4 (8).



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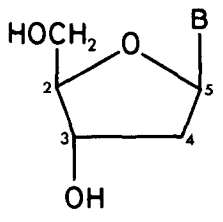

9.

pseudequatorial.

ACKNOWLEDGEMENT

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REFERENCES

1. P. G. Waser, Pharmacol. Rev., 13, 465 (1961).
2. R. W. Baker, C. Chothia, P. Pauling and T. J. Petcher, Nature, 230, 439 (1971); D. J. Triggle and C. R. Triggle, Chemical Pharmacology of the Synapse, Academic Press, New York 1976, p 234.

Table 4 ^3J(HH) magnitudes (Hz) in Muscarine and Related Compounds

	$J_{2,3}$	$J_{3,4}$	$J_{3,4'}$	$J_{4,5}$	$J_{4',5}$
Muscarine	3.0	2.4	5.2	7.0	9.2
β-2'-deoxypseudouridine[a]	2.1	3.2	5.4	7.0	9.0
thymidine-5' phosphate[b]	3.0	2.6	6.6	6.2	7.6
deoxycytidine-5' phosphate[b]	3.2	4.0	6.0	6.3	7.0
deoxyuridine-5' phosphate[b]	3.0	3.0	6.5	6.3	7.5

[a] R P L Conrad and D M Brown, unpublished work.
[b] from D B Davies (Ref 12).

It is evident from Table 4 that there is a remarkably good correlation in J values between the ring protons of muscarine and those of the C-nucleoside 2'-deoxypseudouridine (2; B = Uracil-5).[19] The $J_{4,5}$ and $J_{4',5}$ values for muscarine are higher than the corresponding coupling constants of the N-nucleotides, (the deoxyribosylring has been renumbered as in 9 for easier comparison with that of muscarine), but these must be corrected for the substituent effect (hetero-cyclic ring N → C; 0.8 Hz) at C(5) of muscarine, in addition to the correction factor of 1.1 Hz for the deoxy-system referred to above.

Using the corrected coupling constant values ($J_{4',5}$,7.3; $J_{2,3}$,3.0 Hz)

$$K_{eq} = J_{4',5}/J_{2,3} = 2.43$$

This corresponds to an equilibrium 71% in favour of the C(4)-endo conformation in muscarine and is in good agreement with the crystal structure of the alkaloid.

In summary, an analysis of coupling constant data reveals that no dramatic changes of ring puckering and orientation of the exocyclic 'onium group occur in going from the crystalline to the solution state. By adopting the C(4)-endo conformation the sterically demanding interaction of the trimethylammonio group with the ring is minimised by making it

3. F. Jellinek, Acta Cryst., 10, 277 (1957)
4. F. G. Canepa, P. J. Pauling and H. Sorum, Nature, 210, 907 (1966).
5. C. C. J. Culvenor and N. S. Ham, Chem. Commun., 537 (1966).
6. D. L. de Fontaine, B. Ternai, J. A. Zupan, R. S. Givens and R. A. Wiley, J. Med. Chem. 21, 715 (1978)
7. A. M. Mubarak and D. M. Brown, Tetrahedron Letters, 21, 2453 (1980).
8. M. Remin and D. Shugar, Biochem. Biophys. Res. Comm., 48, 636 (1972).
9. F. E. Hruska, A. A. Grey and I. C. P. Smith J. Amer. Chem. Soc., 92, 4088 (1970); R. H. Sarma, R. J. Mynott, D. J. Wood and F. E.Hruska, J. Amer. Chem. Soc., 95, 6457 (1973).
10. C. Altona and M. Sundaralingam, J. Amer. Chem. Soc., 95, 2333 (1975)
11. D. B. Davies and S. S. Danyluk, Biochemistry, 13, 4417 (1974).
12. D. B. Davies, Prog. in NMR Spectrosc., 12, 135 (1978).
13. S. S. Danyluk in Nucleoside Analogues, Chemistry, Biology and Medical Applications Ed. R. T. Walker, E. de Clercq and F. Eckstein, Plenum Press, London 1971 p 15.
14. H. Booth and G. C. Gidley, Tetrahedron, 21, 3429 (1965).
15. A. F. Casy, NMR Spectroscopy in Medicinal and Biological Chemistry, Academic Press, London 1971
16. R. J. Abraham and G. Gatti, J. Chem. Soc. B, 961 (1969).
17. E. M. Nottoli, J. B. Lambert and R. L. Letsinger, J. Amer. Chem. Soc., 99, 3486 (1977).
18. S. Wolff and W. C. Agosta,J. Org. Chem., 45, 1332 (1980).
19. D. M. Brown and R. P. L. Conrad, unpublished work.